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(19) (CA) **CANADIAN PATENT** (12)

(54) Antimicrobial Composition

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ABSTRACT

An aqueous antimicrobial composition comprising from about 10 to about 30 percent by weight of ethyl alcohol, from about 2 to about 5 percent by weight of benzyl alcohol and the remainder to 100% water, and a method of use of the composition for destroying or reducing the number of microbes on a surface contaminated therewith.

ANTIMICROBIAL COMPOSITION

The invention relates to aqueous ethanolic antimicrobial compositions for treating surfaces so as to render them essentially free of harmful microbial contamination or reduce the level of harmful microbial contamination thereon.

It is well known that ethyl alcohol, in combination with water, at concentrations of 50-90% provides broad spectrum germicidal activity. Aqueous ethyl alcohol (70%) is also known to be virucidal against lipophille and some hydrophilic viruses. Hard surface disinfectant compositions based on aqueous ethyl alcohol also are known.

Benzyl alcohol is also known to possess antibacterial and antifungal activities. U.S. Patent 4,200,655 discloses that benzyl alcohol possesses virucidal activity at concentrations in the range of from 1.5 to 6%.

U.S. Patent 4,695,453 discloses thickened alcoholic antibacterial compositions 40 and 50% by weight of ethanol which contains 4% water, a minor portion (between 20% and 30% by weight) of anhydrous isopropanol, and a nominal amount (0.5% to 2% by weight) of benzyl alcohol.

The problem is that such previously disclosed compositions containing 40% or more ethanol are flammable.

The above problem is overcome by the present invention which provides an aqueous antimicrobial composition comprising from 10 to 30 percent by weight of ethyl alcohol, from 2 to 5 percent by weight of benzyl alcohol.

The composition of this invention has a lower flashpoint than prior art compositions and at the same time unexpectedly exhibits synergistic antimicrobial activity with respect to particular pathogenic organisms as more fully discussed herein below. Moreover, the composition exhibits broad spectrum antibacterial activity, antiviral activity and tuberculoidid



activity. The broad spectrum activity is achieved in a rapid 10 minute surface contact time.

The compositions of the invention are useful for destroying or reducing the number of disease or other harmful microorganisms on a variety of surfaces contaminated therewith thereby essentially eliminating or significantly reducing the potential for spreading diseases associated with the microorganisms through contact with the contaminated surfaces.

The concentration in the composition of ethyl alcohol is from 10 to 30 weight percent, preferably from 19 to 23 weight percent, and of benzyl alcohol is from 2 to 5 weight percent. More preferably the concentration of ethyl alcohol is from 19 to 21 weight percent and of benzyl alcohol is from 3 to 5 weight percent.

The major component of the compositions of the invention is water, the concentration of which, based on the total weight of the two alcohols and other optional ingredients, ranges from 65 to 88 weight percent.

One or more other ingredients may optionally be included in the compositions of the invention in order to provide aesthetic or other beneficial properties thereto. Such optional ingredients are, for example, additional antimicrobial agents, deodorizers, emulsifiers, solubilizers, corrosion inhibitors when the compositions are packaged in metal containers, e.g., aerosol containers, and solvents, the only requirement being that for any particular composition such optional ingredients be compatible with the other ingredients present therein.

By way of example, optional ingredients which may be incorporated include the following:

Antimicrobials - phenolic compounds such as o-phenylphenol, o-benzyl-p-chlorophenol and 4-tert-amylphenol; and quaternary ammonium compounds such as alkyl dimethyl benzyl ammonium chloride, octyl decyl dimethyl ammonium chloride, dioctyl dimethyl ammonium

chloride, didecyl dimethyl ammonium chloride and alkyl dimethyl benzyl ammonium saccharinate.

Deodorizer - N-alkyl-N-ethylmorpholinium ethyl sulfate.

5 Emulsifier/Solubilizer - lauryl dimethyl amine oxide, polyoxypropylenepolyoxyethylene block copolymer and anionic, cationic and nonionic surfactants.

Corrosion Inhibitor - mono- and triethanolamine, ammonium hydroxide, sodium molybdate, sodium benzoate
10 and tetra sodium ethylenediamine tetraacetate (Na₄EDTA).

Solvent - alcohols such as isopropyl alcohol and butyl alcohol, each of which can also contribute to antimicrobial activity.

15 The amounts of optional ingredients to be employed can readily be determined by one skilled in the art. For example, the phenolic and quaternary ammonium antimicrobial agents generally will not exceed a concentration of 0.2 percent by weight.

20 The compositions of the invention may be formulated with conventional propellants for dispensing as aerosols from conventional pressurized containers. Propellants which may be used are well known and conventional in the art and include, for example,
25 isobutane, n-butane, propane, dimethyl ether and blends thereof as well as individual or mixtures of chlorofluoro- and/or fluorohydrocarbons. The amount of propellant employed should provide a suitable spray pattern and for essentially complete expulsion of the
30 composition from the aerosol container. The appropriate amount to be used for any particular aerosol propellant system can readily be determined by one skilled in the art. Generally speaking, the amount of a particular propellant employed should provide an
35 internal pressure of from 30 to 100 p.s.i.g.

In order to realize the full antimicrobial potential of the compositions of the invention, the contact time of the composition with the microorganism should be at least 10 minutes.

The compositions can be packaged in conventional, ready-to-use dispensing systems. Thus they can be packaged in aerosol form in conventional aerosol containers or in liquid form in trigger pumps spray
5 bottles and squeeze bottles. They can also be impregnated into towelettes and packaged individually or packaged in bulk form for individual dispensing.

The compositions can be prepared by entirely conventional procedures, no special techniques being
10 required. They are conveniently prepared by adding the ethyl alcohol and the benzyl alcohol to water with mixing followed by any optional ingredients.

The compositions of the invention are illustrated by examples of specific formulations described
15 below without, however, being limited thereto. The concentration of ethyl alcohol in all formulations is based on 100% active. Deionized water was employed in all formulations.

<u>Ingredient (% by weight)</u>			
<u>Example</u>	<u>Ethyl Alcohol</u>	<u>Benzyl Alcohol</u>	<u>Water</u>
1	10	2	88
2	10	3	87
5 3	10	3.5	86.5
4	10	4	86
5 5	20	2	78
6	20	3	77
7	20	4	76
10 8	25	2	73
9	30	3	67
10 10	30	4	66

The compositions of Examples 2, 3, 4, 6 and 8 were tested for antiviral activity against Rhinovirus Type 39 using Method I below. The components of Examples 1, 2, 5, 7, 9 and 10 were tested for antibacterial activity against Staphylococcus aureus (ATCC 6538) using test method II below.

20 Method I

The virus stock was propagated in a continuous line of HeLa(Ohio) cells, and generally contained loglo 6.0 TCID₅₀/ml- For testing , 0.2 ml of virus stock (containing 10% inactivated fetal calf serum (IFCS)) is spread over the surface of a 60 mm petri plate. The plate is then placed at 35°C for 45 minutes to dry the inoculum to a uniform film. The test agent (2.0 ml) is then applied to the virus film and allowed to remain in contact, at room temperature, for 10 minutes. After the 10 minute contact time, serial ten-fold dilutions are made in assay medium (BME + 2% IFCS) and 0.2 ml of each dilution of virus/test agent is then placed into each of four separate wells of MRC-5 cells (grown in BME + 2% IFCS). The assay plates are incubated at 33°C for 10-14 days, with media changes every 3-4 days. Plates are scored for characteristic viral cytopathic effect (cellular rounding and degeneration).

Method II

Horse serum is added to a culture of the bacteria to achieve a final concentration of 5% and 0.1 ml of this bacterial suspension is inoculated onto a glass petri plate.

The inoculum is spread into a 0.5 inch diameter circle and allowed to dry for 30 to 40 minutes at 37°C and treated with 1.0 ml of the test agent. After 10 minutes contact time, 9 ml of letheen broth is added to the plate and the surface of the plate is swabbed to loosen any remaining bacteria. Serial dilutions of the test sample are made and plated out to 10⁻¹, 10⁻³, and 10⁻⁵. The test agent is treated in duplicate at each dilution. The assay plates are incubated for 48 to 72 hours at 37°C, the number of colonies at each dilution are counted and the average of the number of colonies from the duplicate tests is calculated for each dilution.

The results of the tests against Rhinovirus Type 39 and Staphylococcus aureus, expressed in terms of logs of inactivation of Rhinovirus Type 39 and Staphylococcus aureus where log 1 or greater represents a 90% or greater kill of the microorganism, log 3 or greater representing a kill of 99.9% or greater, are given in Table A.

Table ALogs of Inactivation

<u>Composition</u>	<u>Staph. aureus</u>	<u>Rhinovirus Type 39</u>
Example 1	3.78	
30 Example 2	5.02 ^a	≤ 1.83
Example 3		2.67
Example 4		3.33
Example 5	5.02 ^a	
Example 6		3.92
35 Example 7	5.02 ^a	
Example 8		≤ 2.0
Example 9	5.43 ^b	
Example 10	5.02 ^a	

a) In Method II, the maximum log of inactivation which could be determined in this particular experiment was 5.02. Thus the log value actually may be higher than indicated.

5 b) This is the result obtained in a repeat test; in the first test a log of inactivation of 4.28 was obtained but this result is believed to be due to experimental error.

10 Compositions consisting of 2, 3 and 4 weight percent benzyl alcohol in water and 10, 20 and 30 weight percent ethyl alcohol in water were tested for antiviral and antibacterial activity against Rhinovirus Type 39 and Staphylococcus aureus (ATCC 6538) using the
15 test procedures of Methods I and II described hereinbefore. The results of these tests, expressed as logs of inactivation, are given in Table B.

Table B

		<u>Logs of Inactivation</u>	
<u>Composition</u>		<u>Staph. aureus</u>	<u>Rhinovirus Type 39</u>
20	2% Benzyl alcohol in water	0.89	0.17
	3% Benzyl alcohol in water	5.02 ^a	0.5
25	4% Benzyl alcohol in water	4.37	≤2.0
	10% Ethyl alcohol in water	0	1.0
	20% Ethyl alcohol in water	0	1.17
30	30% Ethyl alcohol in water	0.72	1.89

a) see footnote a), Table A

35

A comparison of the test results in Tables A and B shows that the combination of ethyl alcohol and benzyl alcohol in the compositions of the invention can provide antiviral activity against Rhinovirus Type 39

and antibacterial activity against *Staphylococcus aureus* the total effect of which is greater than the sum of the two effects taken independently. This is best demonstrated in the case of *Staphylococcus aureus* by Examples 1, 5 and 7 where the total effect in logs of inactivation versus the additive effects is respectively 3.78 v. 0.89, 5.02 v. 0.89 and 5.02 v. 4.37; and in the case of Rhinovirus Type 39 by Examples 2, 4 and 6 where the total effects versus the additive effect is respectively 1.83 v. 1.5, 3.33 v. < 3.0 and 3.92 v. 1.67.

The following compositions were formulated as concentrates for aerosol dispensing.

Example	% by Weight			
	11	12	13	14
<u>Ingredient</u>				
Ethyl alcohol	20.00	20.00	19.40	22.70
Benzyl alcohol	4.00	4.00	5.00	2.00
o-Phenylphenol	0.20	-	-	0.10
Quaternary ammonium compound	-	0.0ga	-	-
Sodium molybdate	0.14	0.14	0.14	0.14
Ammonium hydroxide (28%)	-	-	0.05	0.05
Water	-----g.s. to 100%-----			

a) 0.02 weight-% Cynical 80%, (Hilton-Davis) (alkyl dimethyl benzyl ammonium chloride wherein alkyl represents 50% $C_{14}H_{29}$, 40% $C_{12}H_{29}$ and 10% $C_{16}H_{33}$); and 0.03 weight-% BTC 818 (Stepan Chemical Company) (octyl decyl dimethyl ammonium chloride (25%), dioctyl dimethyl ammonium chloride (12.5%) and didecyl dimethyl ammonium chloride (12.5%)).

The compositions of Examples 11, 12, 13, and 14 were formulated and packaged for dispensing as aerosols (Examples 11A, 12A, 13A and 14A respectively). In each case the concentrate consisted of 71 percent by weight of the composition and 29 percent by weight of a

hydrocarbon propellant consisting of 80 parts of isobutane and 20 parts of propane.

The aerosol formulations of Examples 11A, 12A, 13A and 14A were tested for antimicrobial activity against *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 15442) in the AOAC germicidal spray test (Official Methods of Analysis of the AOAC, 14th ed., 1984, page 71). Thirty slides were tested for bacterial growth per sample per microorganism. The test results, expressed in terms of the number of slides of 30 slides showing bacterial growth, are given in Table C.

Table C

<u>Microorganism</u>	<u>Example:</u>	<u>11A</u>	<u>12A</u>	<u>13A</u>	<u>14A</u>
15 <i>Staph. aureus</i>		0/30	0/30	0/30	1/30
<i>Ps. aeruginosa</i>		0/30	1/30	0/30	0/30

The results in Table C demonstrate the antimicrobial effectiveness of the aerosol formulations of Examples 11A, 12A, 13A and 14A against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The aerosol formulations of Examples 11A, 12A, 13A and 14A were tested against Rhinovirus Type 39 using the following test method:

25 Method III

The virus stock was propagated in a continuous line of HeLa (Ohio) cells, and generally contained log₁₀ 6.0 TCID₅₀ per 0.2 ml. For testing, 0.2 ml of virus (containing 5% inactivated newborn calf serum (INCS)) is spread over the surface of a 60 mm petri plate. The plate is then placed at 35°C for 45 minutes to dry the inoculum to a uniform film. The spray can containing the test agent is held 6-8" from the surface of the plate at an angle of 45°, and the spray is expelled for 3 seconds (2 ml expelled volume). After a 10-minute contact time, serial tenfold dilutions of the treated virus are carried out in growth medium (EMEM + 5% INCS). Growth medium is removed from the wells of 12 well assay plates containing subconfluent HeLa

(Ohio) cells, and replaced by 2 ml of maintenance medium (EMEM + 2% INCS). A 0.2-ml aliquot of each virus/test agent is then placed into each of four separate wells. The assay plates are incubated at 33°C for 10-14 days, with media changes every 3-4 days. Virus controls are carried out in an identical manner with 2 ml of serum-free EMEM applied to the virus film in place of the spray treatment. Cytotoxicity controls are carried out by repeating the above procedure with 0.2 ml of growth medium as the initial inoculum. Plates are scored for characteristic viral cytopathic effect (cellular rounding and degeneration).

The results of the antiviral test, expressed as logs of inactivation and percent kill of the microorganism, are given in Table D.

Table D

<u>Example</u>	<u>% Kill</u>	<u>Logs of Inactivation</u>
11A	> 99.99	≥ 4
12A	> 99.9	≥ 3
13A	> 99.99	≥ 4
14A	> 99.9	≥ 3

The results in Table D demonstrate the antiviral effectiveness of the aerosol formulations of Examples 11A, 12A, 13A and 14A against Rhinovirus Type 39.

Compositions of the invention were formulated as concentrates for aerosol dispensing (Examples 15 and 16) and for pump-spray dispensing (Examples 17 and 18) as follows:

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		% by weight			
		Example: 15	16	17	18
Ingredient					
	Ethyl alcohol	21.00	20.00	19.01	19.01
5	Benzyl alcohol	3.00	4.00	3.0	3.0
	o-Phenylphenol	0.10	-	0.075	-
	Quaternary ammonium compound	-	0.05a	-	0.05a
	N-Alkyl-n-ethylmorpholinium ethyl sulfate (35%)b	0.17	0.17	0.12	0.12
10	Lauryl dimethyl amine oxide (40%)c	-	-	0.2	0.2
	Triethanolamine (99%)			0.2	0.2
	Polyoxypropylene-polyoxyethylene block copolymer	-	-	0.75	0.75
15	Sodium benzoate	0.30	0.30	-	-
	Ammonium hydroxide (28%)	0.05	0.035	0.1	0.1
	Fragrance	0.14	0.14	0.12	0.12
	Water	-----g.s. to 100%-----			

- a) 0.02 weight percent of Cyncal 80%, and 0.03 weight-% of BTC 818 [see footnote a), Examples 11-14].
- b) Atlas™ G-271 (ICI Americas); CTFA name: soyaethyl morpholinium ethosulfate.
- c) Ammonyx™ DMCD-40 (Stepan Chemical Company); CTFA name; lauramine oxide.
- 25 d) Pluronic™ F-127 (BASF-Wyandotte); CTFA name: Poloxamer 407.

The compositions of Examples 15 and 16 were formulated and packaged for dispensing as aerosols (Examples 15A and 16A respectively). In each case the concentrate consisted of 71 percent by weight of the composition and 29 percent by weight of a hydrocarbon propellant consisting of 80 parts of isobutane and 20 parts of propane.

35 The aerosol formulations of Examples 15A and 16A and the compositions of Examples 17 and 18 were tested for antimicrobial activity by standard methods.

In the case of bacteria and fungi, the formulations were tested in the AOAC germicidal spray test (Official Methods of Analysis of the AOAC, 14 ed., 1984, page 71). In the case of viruses, testing was performed using Method I described hereinbefore except that for each virus tested, the appropriate host cell line, assay medium and temperature were employed as follows:

	<u>Virus</u>	<u>Host Cell</u>	<u>Medium</u>	<u>Temp (C)</u>
10	Rhinovirus Type 39	MRC-5	BME+2% IFCS	33°
	Herpes Simplex 1 & 2	Vero	199+2% IFCS	37°
	Vaccinia	Vero	199+2% IFCS	37°
	Adenovirus Type 2	293	199+2% IFCS	37°
	Respiratory Syncytial	HET-2	199+2% IFCS	37°
15	Influenza A2 & B	MDCK	199+10ug/ml trypsin	37°
	Human Rotavirus	MA-104	199+5ug/ml trypsin	37°

20 These tests showed that the aerosol formulations of Examples 15A and 16A and the compositions of Examples 17 and 18 provided greater than 99.9% kill in each case against the following microorganisms:

25	<u>Viruses</u>	<u>Bacteria</u>
	Rhinovirus Type 39	
	Herpes Simplex 1&2	Pseudomonas aeruginosa
	Human Rotavirus	Staphylococcus aureus
	Respiratory Syncytial	Salmonella choleraesuis
30	Influenza A2 & B	
	Adenovirus Type 2	
	Vaccinia	

	<u>Fungi</u>
35	Aspergillus niger
	Trichophyton mentagrophytes

The composition of Example 17 also was shown to provide greater than 99.9% kill of Mycobacterium

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tuberculosis var. bovis when tested by the AOAC
tuberculocidal method (Official Methods of Analysis of
the AOAC, 14th ed., 1984, page 73).

Claims:

1. An aqueous antimicrobial composition comprising ethyl alcohol and benzyl alcohol characterized in that the composition comprises in combination from about 10 to about 30% by weight of ethyl alcohol, from about 2 to about 5% by weight of benzyl alcohol , and water.
2. A composition according to Claim 1 wherein the amount of ethyl alcohol is from 19 to 21% by weight and of benzyl alcohol is from about 3 to about 5 percent by weight.
3. A composition according to claim 1 or 2 that includes up to about 0.2% by weight of an antimicrobial agent selected from the group consisting of phenolic compounds and quaternary ammonium compounds.
4. A composition according to claim 3 wherein the antimicrobial agent is o-phenylphenol.
5. A composition according to claim 4 wherein the o-phenylphenol is present in an amount of from about 0.07 to about 0.2 percent by weight.
6. A composition according to claim 3 comprising about 19% by weight of ethyl alcohol, 3% by weight of benzyl alcohol and 0.12% by weight of o-phenylphenol.
7. An aqueous antimicrobial composition in aerosol form comprising a hydrocarbon propellant and an antimicrobial composition according to any one of claims 1, 2, 4, 5 or 6.
8. A composition according to any one of claims 1, 2, 4, 5 or 6, impregnated into a towelette.



SUBSTITUTE
REMPLACEMENT

SECTION is not Present
Cette Section est Absente

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